

## **Age-dependent effects of T-2 toxin and deoxynivalenol on some lipid peroxide and antioxidant parameters in chicken**

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### **Summary**

The aim of this study was to measure the effects of T-2 toxin in a dose of 5.77 mg/kg feed and deoxynivalenol 4.86 mg/kg feed on the antioxidant enzyme system and some lipidperoxidative parameters in two different age-groups of chickens in 72 hours with a frequented sampling in the first 24 hours. The results suggest that, the mycotoxin exposure activated the enzymatic antioxidant system, but in a different way in each age-groups by each mycotoxin. The parameters of the initiation of lipidperoxidation parameters were affected several times, but these were not followed by the levels of malondialdehyde, which suggests that, the antioxidant system was able to compensate the effects of the mycotoxins and protected the liver from the lipidperoxidative damage.

### **1. Introduction and literature review**

Mycotoxins are secondary metabolites, produced by several moulds. The presence of mycotoxins in feed cause impairment of the production traits and toxic effects in animals in a dose dependent way (Diaz, 2005). T-2 toxin and deoxynivalenol (DON) are mycotoxins produced by several species of the genus *Fusarium* mould (Jelinek *et al.*, 1989). DON is one of the most frequently found mycotoxins in cereal grains worldwide. While T-2 toxin have been described in 1968 by Bamberg *et al.* and considered as one of the most toxic trichothecene mycotoxin. T-2 toxin and DON inhibit protein and DNA synthesis in eukaryotic cells (Holladay, 1995). Also, DON acts on the serotonergic system as a neurotoxic effect, resulting in its ability to mediate feeding behaviour and cause an emetic response (Fioramonti *et al.*, 1993) which also may be related to the effects on cell signalling processes (Leathwood, 1987). Chemical structure of T-2 toxin and DON contain an epoxy group, which may play role in their dermatotoxic effects (Szilágyi *et al.*, 1994). Previously, it was found that the intensity of lipid peroxidation processes increased in farm animals as a result of long term exposition of trichothecenes in context of biochemical changes in cells which affect the activity of the biological antioxidant system as well (Mézes *et al.*, 1998; Surai *et al.*, 2002).

During the evolution an efficient enzymatic and non-enzymatic antioxidant defence system has evolved in the biological systems to protect the cells and cell organs against the damage of (per)oxidation (Chow, 1988; Davies, 1995). Glutathione-dependent enzymes play role in detoxification and in enzymatic antioxidant system as well, such as glutathione peroxidases or glutathione peroxidase activity of Alpha-class glutathione S-transferases (Zhao *et al.*, 1999). Polyunsaturated fatty acids are the most sensitive and particularly prone to oxidative damage caused by the reactive oxygen species, however, reactive oxygen species

generated by the peroxidative processes can damage other biomolecules as well. This damage affects mainly the biological membranes which results in reduced fluidity, integrity, and increased permeability (*Gutteridge and Halliwell, 1990*), which can lead to cell death or lysis (*Mézes and Matkovics, 1986*). The chain reaction initiated by the reactive oxygen species can be terminated by scavenging antioxidant molecules (such as reduced glutathione (GSH) or vitamin E) or in enzymatic ways (*Chow, 1988; Davies, 1995*).

## 2. Material and methods

After 1 week acclimatisation 165 one-week-old and another 165 three-week-old ROSS 308 male broiler chicks were randomly assigned to 3 dietary treatments, (control, T-2 toxin and DON), in two replicates in each group. Chicks were maintained on a 24-h continuous light schedule and allowed ad libitum access to feed and water.

The diets were experimentally contaminated with 5.77 mg of T-2 toxin (HT-toxin<0.1 mg/kg) and 4.86 mg of DON/kg feed. According to the guideline for a no observed effect level value of 0.5 mg/kg feed T-2 in poultry as proposed by Eriksen and Pettersson (2004), despite the fact that a maximum recommended value of 0.25 mg/kg feed according to the EU proposal (2013/165/EU) in compound feed. The recommendation for DON (2006/576/EC) sets a limit of 5 mg/kg complete feed.

Liver samples were taken after 0, 2, 4, 8, 12, 16, 20, 24, 36, 48, 60 and 72 hours after toxin exposition of 5 randomly chosen chicken from each group and stored at -70 °C until analysis. The initial sampling was made at 8.00 a.m..

Liver samples were homogenized in 9-fold cold (4°C) physiological saline (0.65% w/v NaCl). Determination of malondialdehyde (MDA) content was carried out in the native homogenate, while the other parameters were determined in the 10,000 g supernatant fraction of the homogenate.

For detecting the initial steps of lipid peroxidation, conjugated dienes (CD) and -trienes (CT) content were measured according to the AOAC (1984) after extraction with trimethylpentane and measuring the absorption at 232 nm and 268 nm, respectively. The meta-stable end product of lipid peroxidation, MDA concentration was measured based on the colour complex formation of malondialdehyde with 2-thiobarbituric acid in an acidic environment at high temperature (*Placer et al., 1966*). The standard was 1,1,3,3-tetraethoxypropane (Fluka, Buchs, Switzerland).

Concentration of reduced glutathione (GSH), and activities of glutathione-peroxidase (GPx) and also glutathione-S-transferase (GST) were measured to observe alterations in the antioxidant system. GSH content of liver homogenate was measured as described by *Rahman et al. (2007)*. GPx activity was determined according to *Lawrence and Burk (1976)*, where the loss of glutathione was measured using Ellmann's reagent (*Sedlak and Lindsay, 1968*). The enzyme activity was expressed as nmol glutathione oxidation per minute at 25 °C. The enzyme activity was calculated to protein content of the 10,000 g supernatant fraction of tissue homogenate, which was measured using Folin-phenol reagent (*Lowry et al., 1951*). GST activity in the liver was measured by an assay kit (Sigma, St.Louis, USA) according to the method described by *Habig et al. (1974)*. GST catalyzes the conjugation of glutathione to CDNB. The product, GSH-DNB conjugate, absorbs at 340 nm. The increase in the absorption is directly proportional to the GST activity in the sample.

Statistical analysis was performed using one-way ANOVA with Tukey-Kramer Multiple Comparison test by GraphPad InStat 3.05 software (GraphPad Software, San Diego, California, USA).

### 3. Results and discussion

Feeding T-2 toxin contaminated diet resulted in significantly elevated GSH concentration after 2, 4, 8, 48, 60 and 72 hours in one week-old chickens (Fig.1), compared to the control while in the three-week-old chickens T-2 toxin also caused significantly elevated GSH concentration after 4, 8 and 20 hours and 36 hours by both mycotoxins and also after 48 h by DON (Fig.2).

T-2 toxin caused significantly elevated GPx activity after 8, 48, and 60 hours in one-week-old chickens (Fig.3), while in three-week-old chickens also significantly elevated levels were observed after 4, 8, 20 hours and also by DON an elevation was measured after 48 h in spite an inhibition was observed after 60 hours in the DON treated group (Fig.4).

In the one-week-old group a significant induction in GST enzyme activity was observed by DON 8 hours after the first toxin exposure, while after 16 hours an inhibition was seen by both mycotoxins (Fig.5). Also a significant activation of GST activity was observed as effect of T-2 toxin exposure after 36 hours in the younger group. In the three-week-old chickens elevated levels were observed by DON after 4 hours and an inhibition by T-2 toxin after 8 hours of the start. Also elevated levels were seen in the T-2 toxin groups after 36 hours and a significant decrease was measured in DON treated group after 72 hours (Fig.6).

These results suggest that, T-2 toxin and deoxynivalenol activated the enzymatic antioxidant system, but in a different way. In the first 24 hours only T-2 toxin caused significant increase of GSH concentration, and consequently enzyme induction in GPx and of the liver, while GST activity was affected by both mycotoxins in both ages. After 24 hours, the older group showed significant changes as an effect of deoxynivalenol in all measured endpoints of the antioxidant system, but in different times, while T-2 toxin exposure resulted in significant changes in the older and younger age-group as well, but also in different exposure-times. The results suggested that the antioxidant status was affected by DON only through the GST enzyme in the one-week-old age during the whole trial, but the older group showed responses in both enzyme levels during the 72 hours of the feeding trial. Thus the different age-groups showed different responses for these trichothecene mycotoxins.

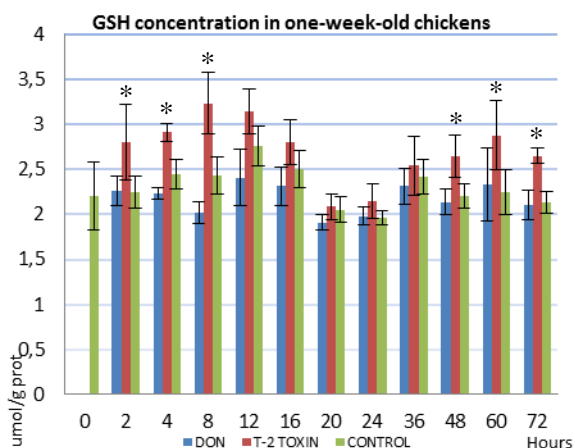


Fig.1: Changes of GSH concentration in one-week-old chickens  
\*p<0.05

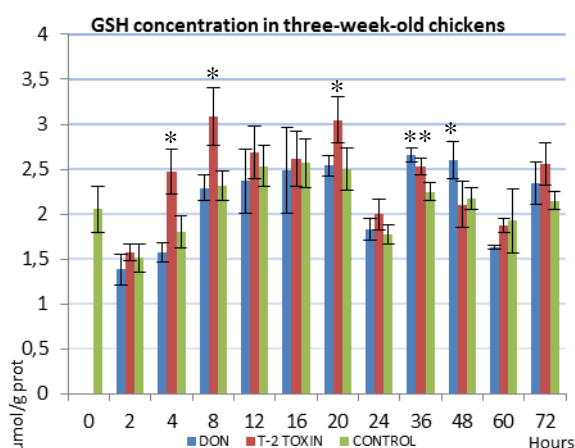


Fig.2: Changes of GSH concentration in three-weeks-old chickens  
\*p<0.05

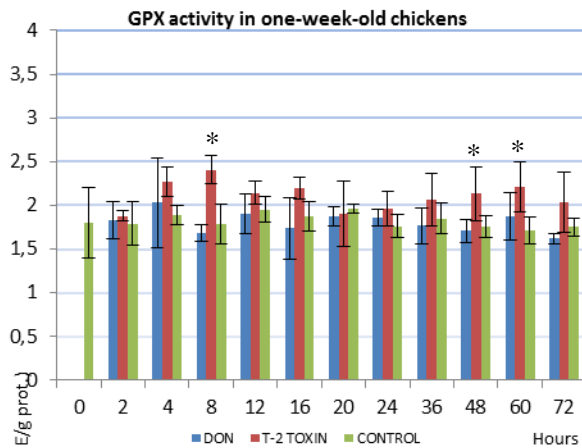


Fig.3: Changes of GPx activity in one-week-old chickens  
\*p<0.05

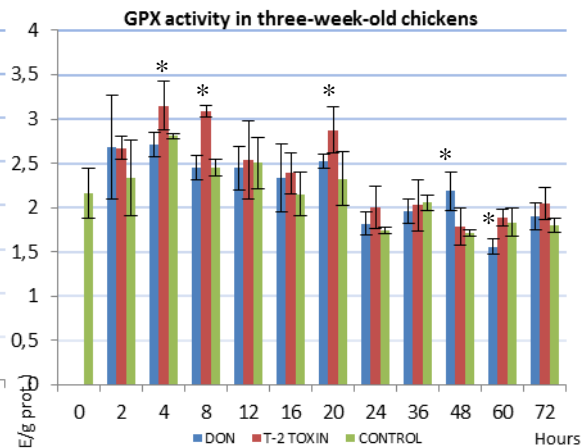


Fig.4: Changes of GPx activity in three-weeks-old chickens  
\*p<0.05

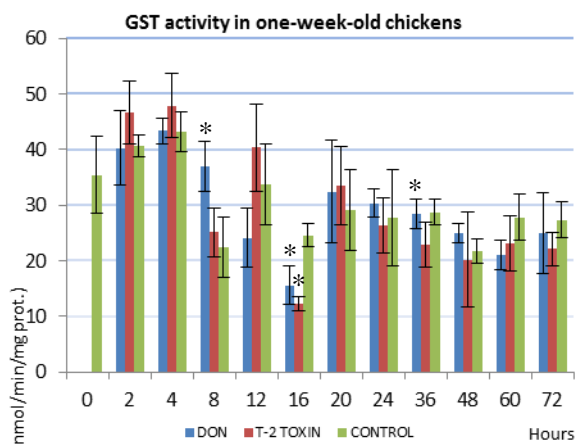


Fig.5: Changes of GST activity in one-week-old chickens  
\*p<0.05

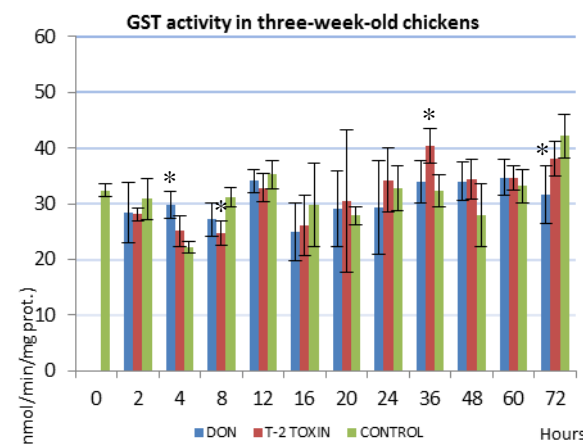


Fig.6: Changes of GST activity in three-weeks-old chickens  
\*p<0.05

In one-week-old chickens CD values elevated in a significant way after 20 hours in the T-2 toxin treated group (Fig.7) in the liver, while elevated levels were observed after 16 and 24 hours as an effect of T-2 toxin, and also lower CD levels were observed after 60 hours by both mycotoxins in the three-week-old chickens (Fig.8).

CT values elevated in a significant way after 20 hours in one-week-old chickens (Fig. 9). In spite, in the three-week-old group CT concentration decreased by DON exposure after 8 and 24 hours and by both mycotoxins after 36 hours (Fig.10).

MDA concentration in the liver was not affected by the mycotoxin exposure in this feeding trial in one-week-old chickens (Fig.11), but in three-week-old chickens MDA concentration was elevated after 8 hours by T-2 toxin, while a decrease was observed by DON exposure after 12 hours (Fig.12).

The end points of lipidperoxidation showed that, significantly affected levels were observed several times in the CD and CT values in the older group, but these were not followed by the increased levels of MDA, which suggests that, the antioxidant system was able to compensate the effects of T-2 toxin and deoxynivalenol, and protected the liver from the oxidative damage. Indeed, the younger age showed less significant changes in the conjugated diene and -triene values, and no significant changes were observed in MDA values, which suggested lower rate of lipidperoxidation. The background of this difference may be related to the faster metabolism of the younger age. Also, significant changes were observed in the first 12 hours of exposition in levels of MDA in the older group, without

previous signs in the conjugated diene and -triene values, which suggests the different rate of lipidperoxidation process, which was restored by the responses of the antioxidant enzyme system afterwards.

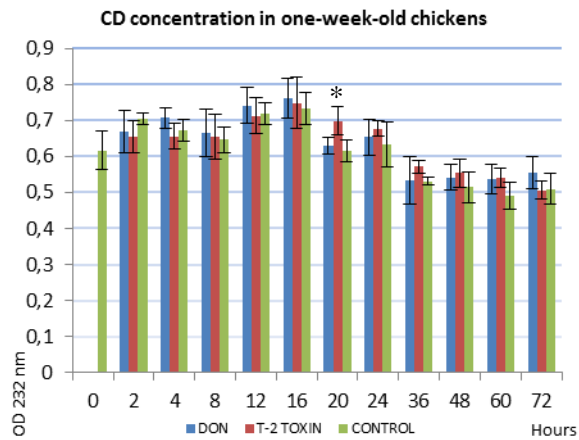


Fig. 7: Changes of CD concentration in one-week-old chickens  
\*p<0.05

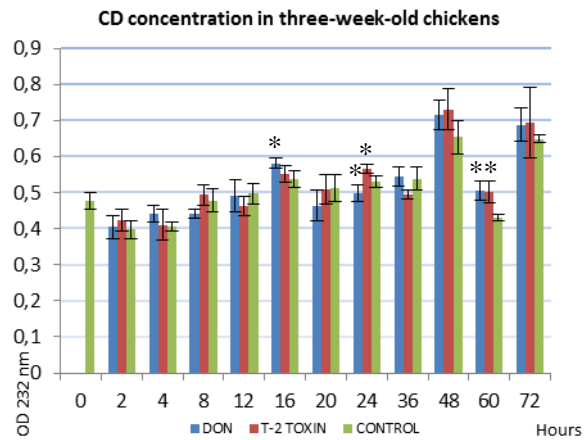


Fig.8: Changes of CD concentration in three-weeks-old chickens  
\*p<0.05

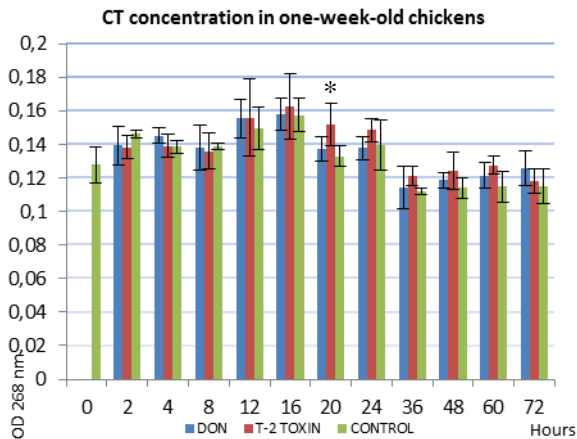


Fig. 9: Changes of CT concentration in one-week-old chickens  
\*p<0.05

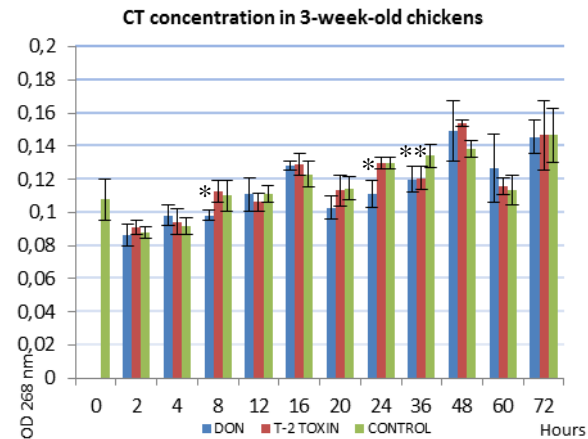


Fig.10: Changes of CT concentration in three-weeks-old chickens  
\*p<0.05

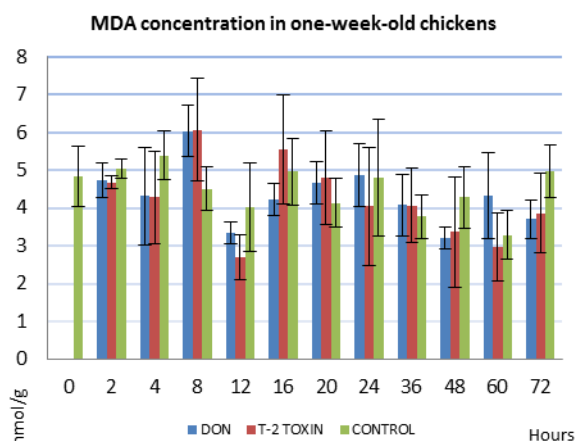


Fig. 11: Changes of MDA concentration in one-week-old chickens  
\*p<0.05

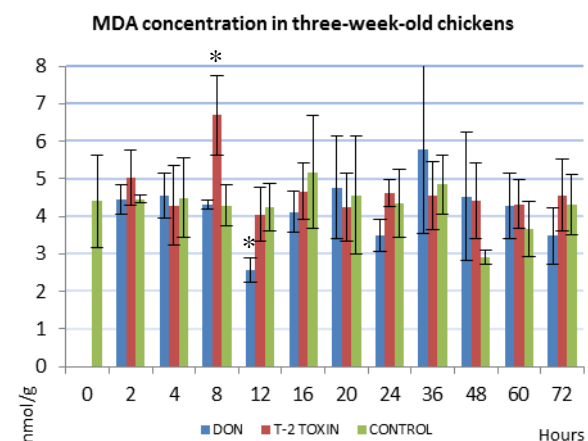


Fig.12: Changes of MDA concentration in three-weeks-old chickens  
\*p<0.05

#### 4. Conclusions

Based on these results we can conclude that, 20-time recommended dose of T-2 toxin and recommended dose of deoxynivalenol resulted different responses of the two age groups

of the chickens in a short, 72 hours, period which may be in association with differences in metabolism and other physiological processes linked to the current age. Otherwise the trends of the effects of the two trichothecene mycotoxins were similar in the two ages. Due to the results, the antioxidant system was able to compensate the effects of T-2 toxin and deoxynivalenol and protected the liver from the oxidative damages in the present trial.

#### Acknowledgement

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